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Double Barrel ESI Source and Novel Tandem NanoLC-MS Setup Enables 24/7 Proteome Profiling with Close to 100% MS Utilization

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PURPOSE

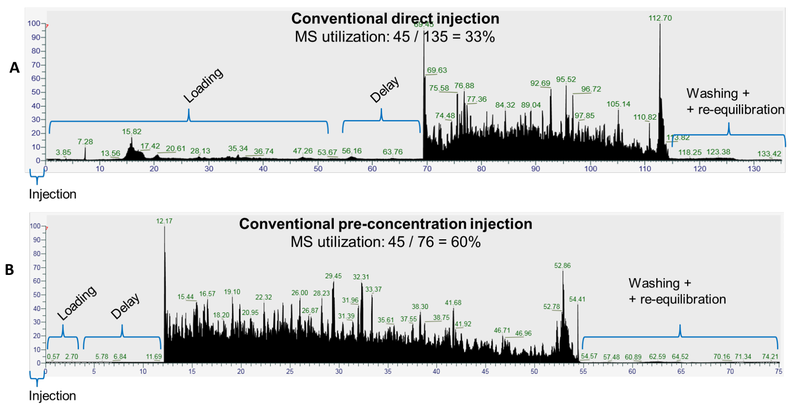
Develop a novel tandem nanoLC-MS/MS platform to maximize MS utilization for high-throughput and deep proteome profiling without compromising separation performance and selectivity.

INTRODUCTION

Nano-flow UHPLC (nanoLC) coupled with high-resolution accurate-mass (HRAM) mass-spectrometry (MS) is the gold standard for deep and quantitative profiling of complex proteomes in discovery proteomics. The unmatched sensitivity of nanoLC-MS, however, is often linked to relatively low MS utilization (the ratio of peptide elution window vs. total run time). The time that is not utilized for the acquisition of useful MS data is needed for sample injection and loading, column washing and equilibration, and sample traveling through the analytical column and fluidics to reach the MS interface (Figure 1).

Here we describe a novel nanoLC-MS platform that eliminates limitations of typical nanoLC-MS setups by combining tandem nanoLC configuration where samples are separated on two independent analytical columns and many loading, equilibration, washing steps can be done in parallel with peptides separation. The new double-barrel ESI source allows simultaneously interface two columns with HRAM MS without post-column flow splitting to maintain the high chromatographic resolution of nanoLC with long columns.

Figure 1. Limited MS utilization in conventional proteomics analysis with direct (A) and pre-concentration (B) injections using nano flow rate (300 nL/min) and long analytical column (75 µm I.D. x 50 cm, 2 µm).



TANDEM NANO LC-MS PLATFORM

The developed tandem nanoLC-MS platform which allows routine 24/7 operation with close to 100% MS utilization comprises **(Figure 2)**:

1) Tandem Thermo Scientific™ UltiMate™ 3000 RSLCnano system;
2) Double Barrel Column Oven (Sonation GmbH) installed onto the Thermo Scientific™ Nanospray Flex™ Ion Source;
3) Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer;
4) Optimized intelligent method script for automated switching between columns during the method execution.

The proposed tandem nanoLC-MS platform can be used for direct injection as well as for preconcentration (trap-and-elute) experiments. Both configuration variants are easy to assemble using fingertight Thermo Scientific™ nanoViper™ fittings and are optimized for maximum separation performance (Figure 3 and 4). The optimized method templates for typical nanoLC-MS proteomics gradients ensure > 95 % MS utilization and high separation quality.

Figure 2. Tandem UltiMate 3000 RSLCnano system coupled with Double Barrel Column Oven and Q Exactive HF-X mass-spectrometer.

Figure 3. Tandem nanoLC direct injection fluidics setup that requires only one 2-position 10-port valve for sample separation on column 1 and column 2.

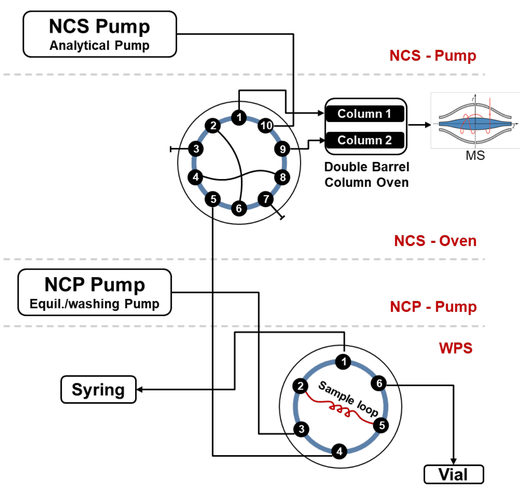
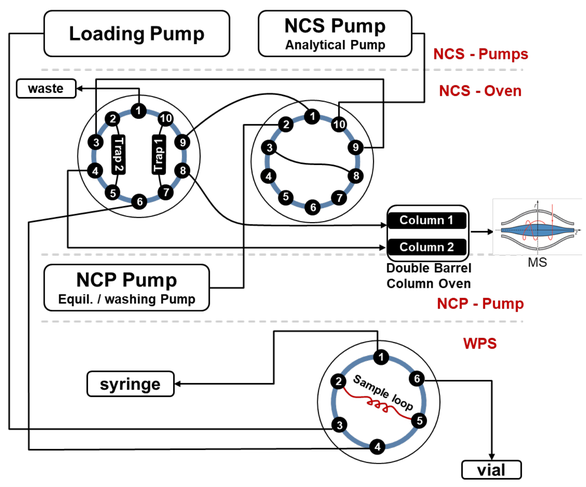


Figure 4. Tandem nanoLC pre-concentration injection fluidics setup that requires two 2-position 10-port valves and loading pump integrated into NCS-3500RS module for sample separation on column 1 and column 2.



METHODS

The tryptic peptides were separated using two analytical self-packed to the emitter columns (75 µm I.D. x 40 cm, C18, Evotec GmbH). The optimized nanoLC-MS methods incorporated "look ahead" injections to load the sample onto the second column while the separation on the first column is still on-going and intelligent automated switching between columns to provide a user experience similar to standard nanoLC-MS sequence setup and execution (Figures 5−8).

Table 1. Tandem nanoLC-MS/MS method details

Attribute	Value
Sample	200 ng Pierce™ HeLa Protein Digest Standard
Column	75 µm ID x 40 cm (C18, 1.9 µm)
Solvent A	0.1% FA-H2O / 0.1% FA-H2O-5%DMSO
Solvent B	0.1% FA-80%ACN / 0.1% FA-80%ACN-5%DMSO
Loading volume	10 µL / 20 µL
Gradient flow rate	250 nL/min
Temperature	60 ºC
Method length	45, 90, 120 min
ESI Voltage application	Liquid junction
MS acquisition	DDA mode
MS1 scan range	m/z 375 to 1500

Figure 5. The operation principle of tandem nanoLC direct injection setup. The NCS-3500RS pump consistently delivers gradient for sample separation on column 1 or column 2 while NCP-3200RS pump and WPS-3000TPL RS autosampler are used for column washing, equilibration, sample injection and loading.

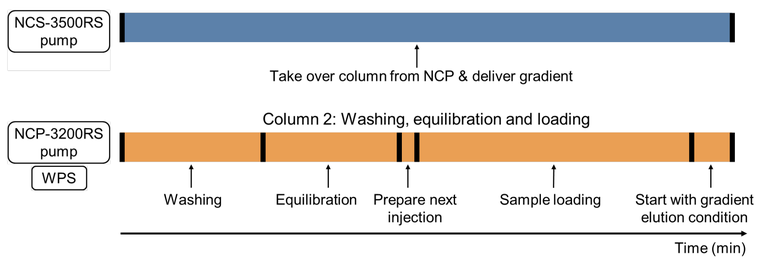


Figure 6. NanoLC gradient profiles on columns 1 and 2 (A) and TIC profiles for HeLa peptides separation on column 1 and 2 (B) for tandem nanoLC direct injection experiments. A single nanoLC-MS/MS method is required to perform peptide separation and MS acquisition on columns 1 and 2 with > 95% utilization. The columns are switched automatically from run-to-run

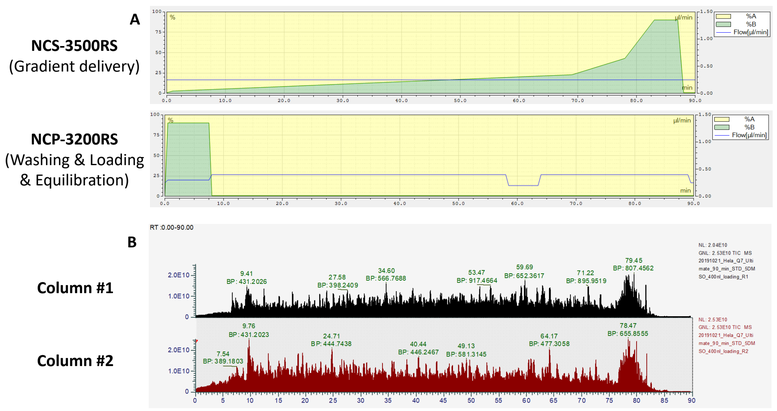


Figure 7. The operation principle of tandem nanoLC pre-concentration injection setup. The NCS-3500RS pump delivers gradient for sample separation on columns 1 or 2, while NCP-3200RS pump, loading pump and WPS-3000TPL RS autosampler are used for offline trap column and analytical column washing and equilibration, sample injection and loading.

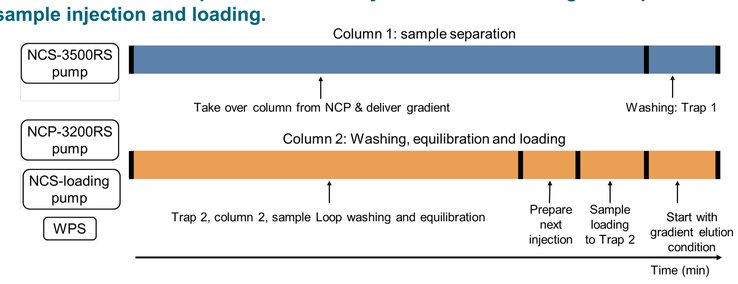
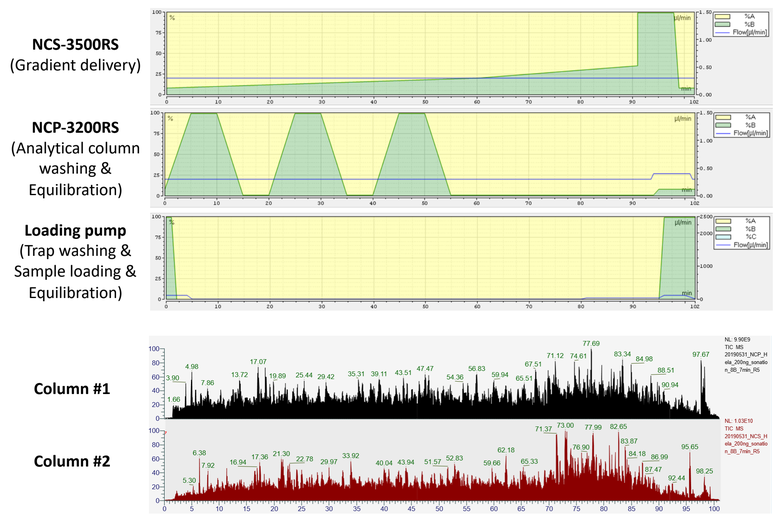


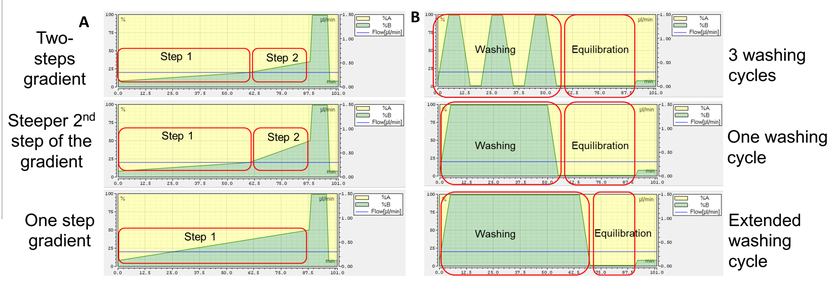
Figure 8.The operation principle of tandem pre-concentration injection setup (A) and TIC profiles for HeLa peptides separation on columns 1 and 2 (B). Additionally to high MS utilization, the pre-concentration setup allows to increase the number of column washing cycles to reduce carry-over and provides faster sample loading that enables shorter nanoLC-MS methods



TANDEM NANO LC-MS VERSATILITY

The tandem nanoLC platform permits users to adjust separation conditions based on the analyzed samples (Figure 9A), e.g., phosphoproteome and TMT-labeled peptides. Additionally, column washing cycles and equilibration volume can be optimized (Figure 9B).

Figure 9. The versatility of tandem nanoLC-MS methods allows to define the number of gradient steps and slopes (A) as well as program washing and equilibration cycles (B).



RESULTS

The tandem nanoLC-MS platform provides MS utilization above 95% with gradients from 45 to 120 min and was tested for 24/7 profiling of complex protein digests. The optimized direct injection nanoLC-MS method with the total runtime of 90-min resulted in the identification of more than >73,000 peptide and >6,700 protein groups in each replicate on column 1 or column 2 (Figure 10). This corresponded to the identification of > 800 peptides during each minute of 24/7 system operation. More than 90% of protein and 80% of peptide groups overlapped between individual runs that shows excellent result reproducibility (Figure 10). Moreover, the high reproducibility of peptide retention on two columns permits precise quantification and low-analytical variability (Figure 11). All the data were processed with Thermo Scientific™ Proteome Discoverer™ 2.4 with 1% false discovery rate (FDR) at protein and peptide levels by using peptide spectral library search followed by iterative Sequest™ HT search engine.

Figure 10. Deep single run HeLa proteome profiling with tandem nanoLC-MS platform using a 90 min direct injection method.

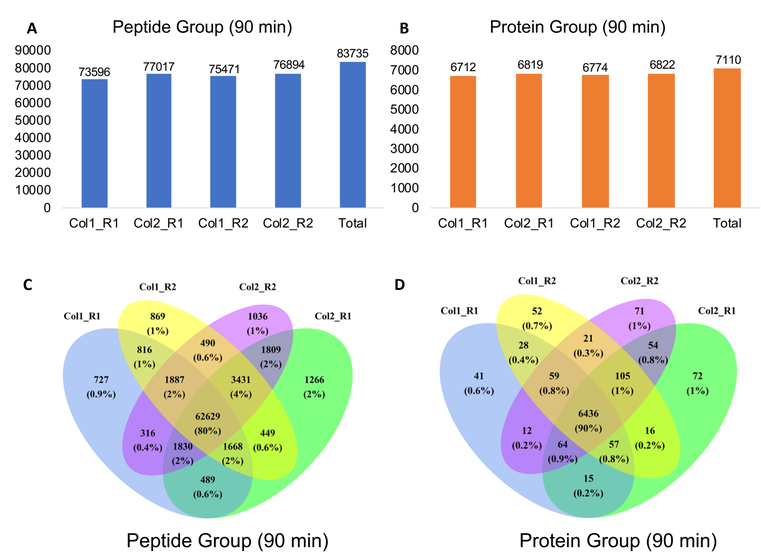
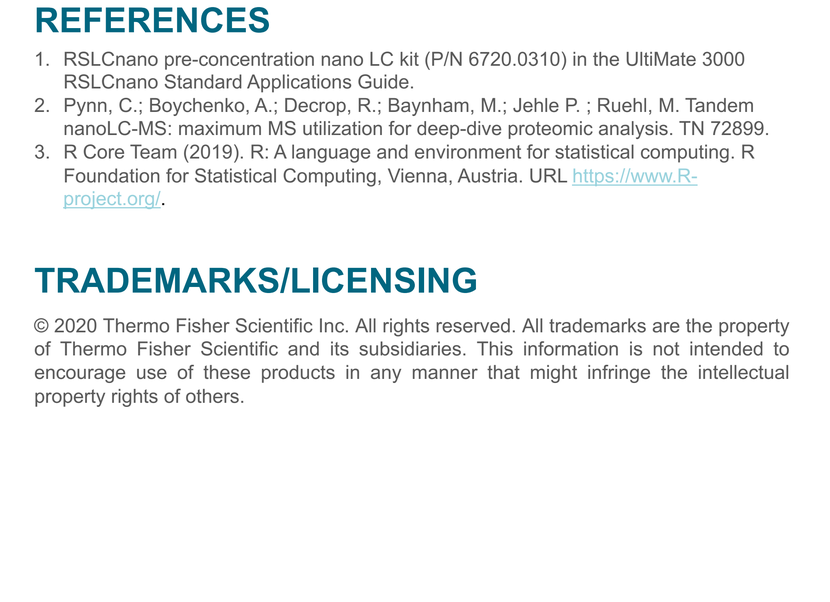


Figure 11. An excellent peptide retention time reproducibility (A, 96% with less than 1 min variation) and low protein abundance variability (B, 74% with less than 25% variation) for tandem nanoLC-MS direct injection analysis of HeLa protein digest.



CONCLUSIONS

We developed a novel tandem nanoLC-MS platform that permits > 95% MS utilization for deep proteome profiling and enables high reproducibility in peptide and protein identification and quantification. The platform could be utilized for sample measurement 24/7 with > 800 peptides identified per each minute of MS operation.

We show how the resolving power of the UltiMate 3000 RSLCnano system coupled with HRAM MS and double barrel ESI source can be combined to create a new standard in the speed and depth of proteome profiling.

The developed approach represents a promising alternative to conventional nanoLC-MS setups for shotgun proteomics as well as targeted analysis in complex matrices.

REFERENCES

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2. Pynn, C.; Boychenko, A.; Decrop, R.; Baynham, M.; Jehle P. ; Ruehl, M. Tandem nanoLC-MS: maximum MS utilization for deep-dive proteomic analysis. TN 72899.
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